

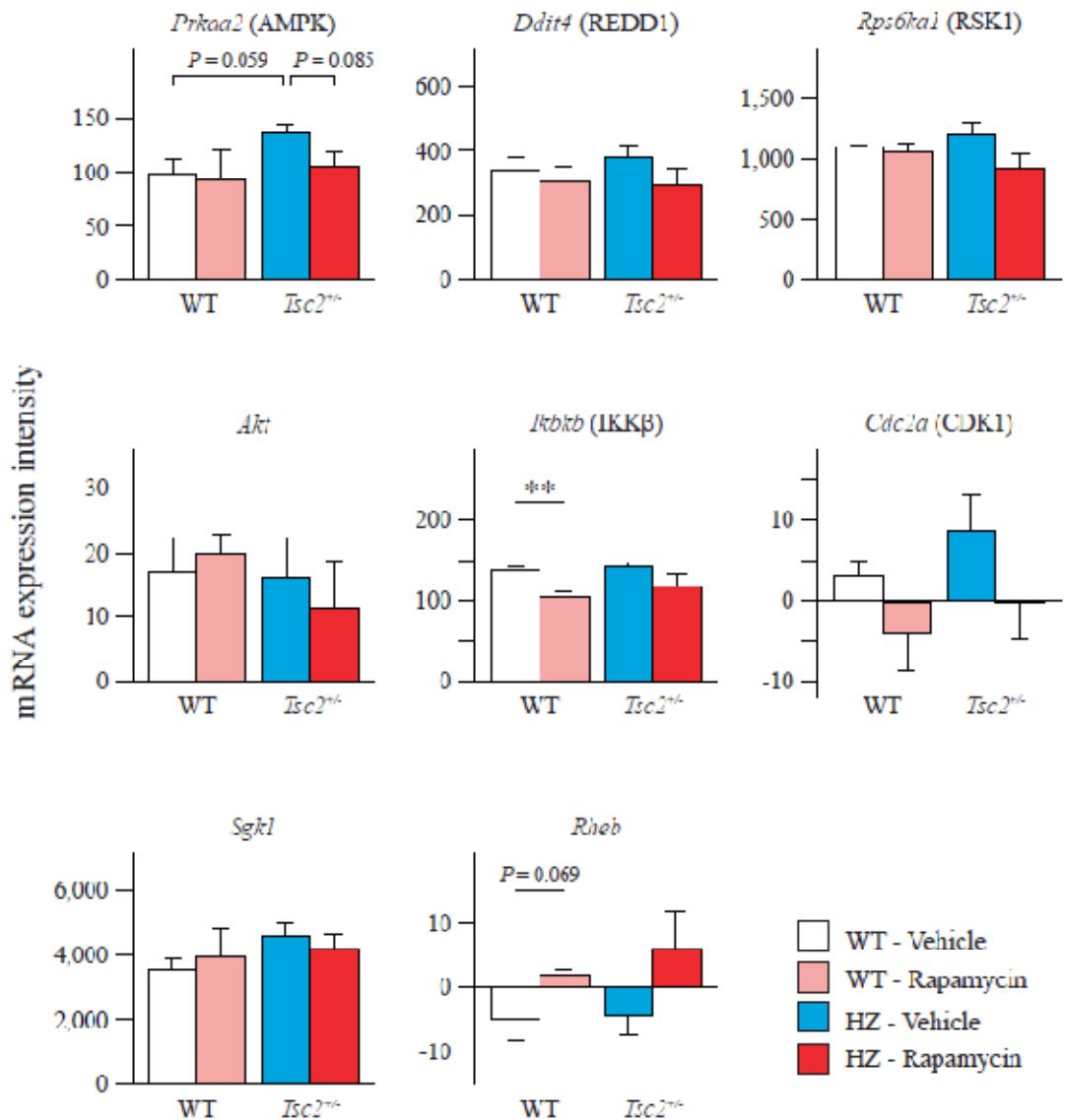
Supplementary Information

Rapamycin reverses impaired social interaction in mouse models of tuberous sclerosis complex

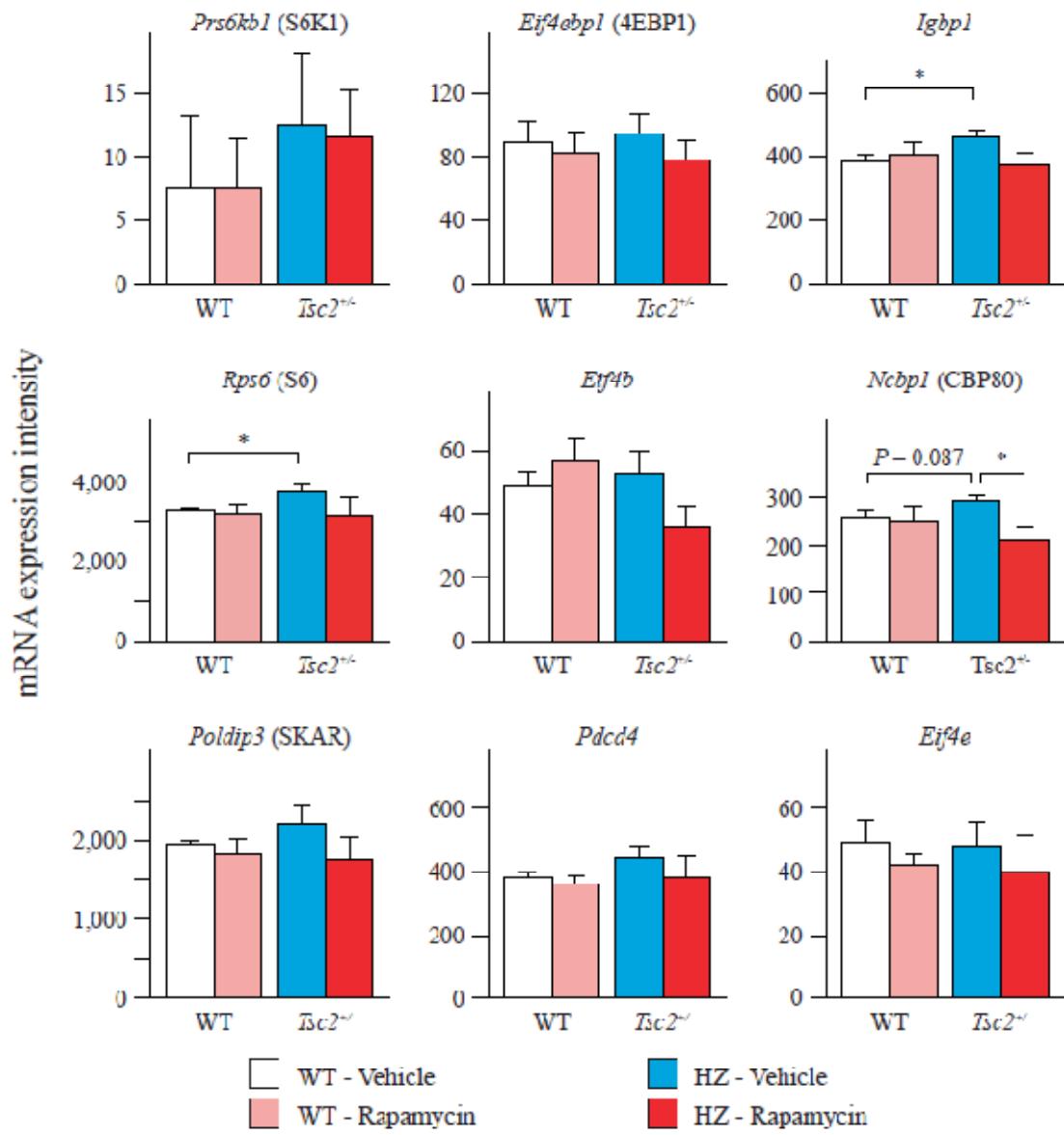
Atsushi Sato, Shinya Kasai, Toshiyuki Kobayashi, Yukio Takamatsu, Okio Hino,

Kazutaka Ikeda, Masashi Mizuguchi

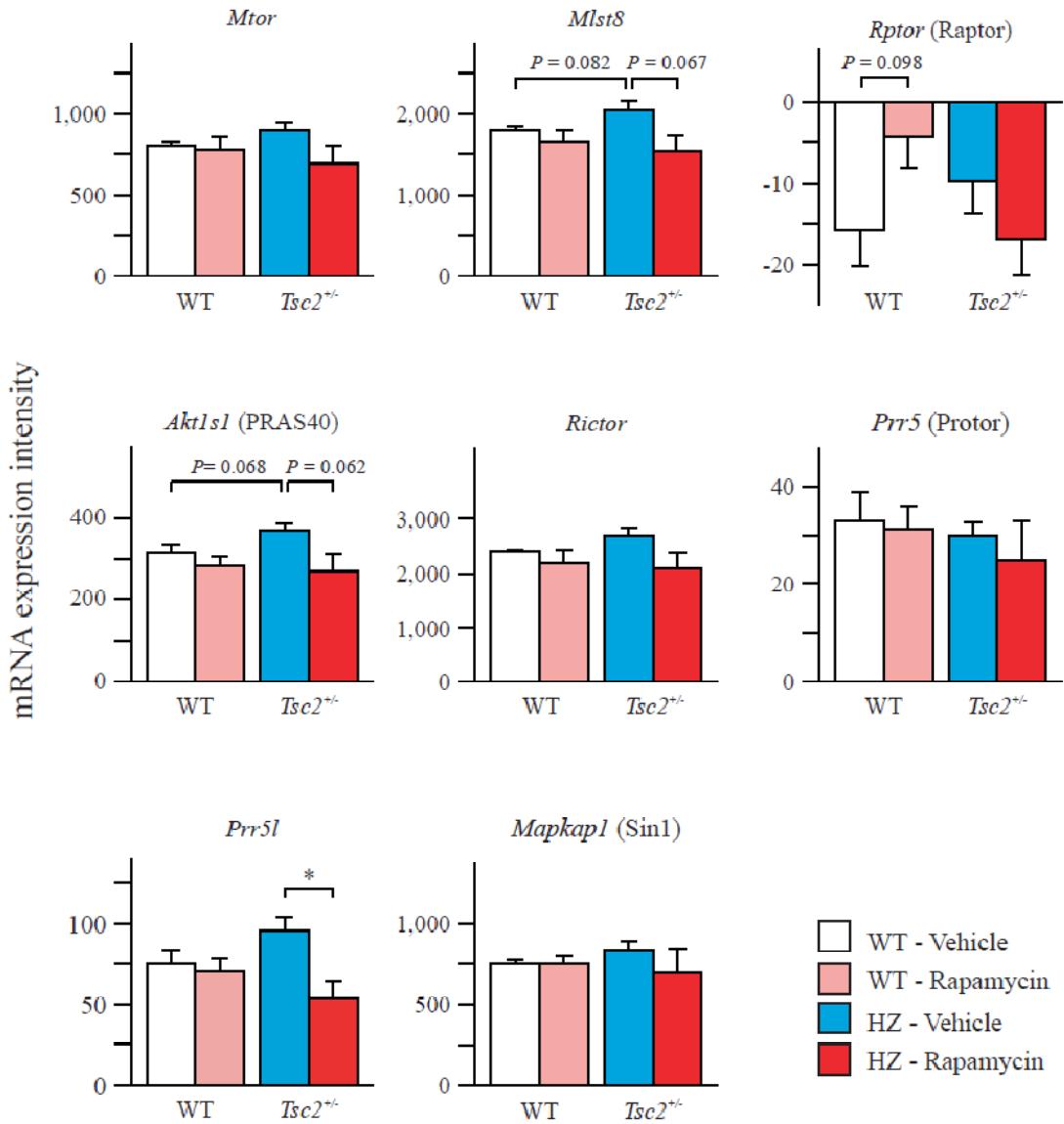
Supplementary Figures



Supplementary Figure S1. Expression of the genes that encode proteins upstream of mTORC1 in the brains of *Tsc2*^{+/-} mice ($n = 4$ for each group) and their controls ($n = 4$ for each group). Error bars indicate s.e.m. ** $P < 0.01$ (two-tailed t -test).



Supplementary Figure S2. Expression of the genes that encode proteins downstream of mTORC1 in the brains of *Tsc2*^{+/-} mice ($n = 4$ for each group) and their controls ($n = 4$ for each group). Error bars indicate s.e.m. * $P < 0.05$ (two-tailed t -test).



Supplementary Figure S3. Expression of the genes that encode the proteins that constitute mTORC1 or mTORC2 in the brains of *Tsc2^{+/-}* mice ($n = 4$ for each group) and their controls ($n = 4$ for each group). Error bars indicate s.e.m. * $P < 0.05$ (two-tailed t -test).

Supplementary Tables

Supplementary Table S1. Tube test for social dominance.

		Wins of mice from	Wins of heavier
		left / matches	mice / matches
<i>Tsc1</i>	Male	16/27 ($P = 0.12$)	9/14 ($P = 0.090$)
	Female	13/30 ($P = 0.29$)	4/10 ($P = 0.38$)
<i>Tsc2</i>	Male	14/26 ($P = 0.27$)	8/15 ($P = 0.30$)
	Female	18/29 ($P = 0.068$)	8/14 ($P = 0.21$)

Supplementary Table S2. General condition, neurological reflexes, and body weight of *Tsc1*^{+/−} mice.

	Male WT (n = 21)	Male <i>Tsc1</i> ^{+/−} (n = 20)	Female WT (n = 24)	Female <i>Tsc1</i> ^{+/−} (n = 20)
Fur condition (%)	100	100	100	100
Bald patches (%)	100	100	100	100
Missing whiskers (%)	100	100	100	100
Piloerection (%)	100	100	100	100
Body tone (%)	100	100	100	100
Limb tone (%)	100	100	100	100
Positional passivity (%)	100	100	100	100
Trunk curl (%)	100	100	100	100
Visual placing (%)	95	100	96	100
Eye blink (%)	100	100	100	100
Ear twitch (%)	100	95	100	100
Whisker twitch (%)	100	100	96	100
Toe pinch (%)	81	85	88	85

	100	100	100	100
Righting reflex (%)	21.3 ± 0.3	20.9 ± 1.0	17.7 ± 0.3	17.8 ± 0.2
Body weight (g)	(<i>n</i> = 22)	(<i>n</i> = 24)	(<i>n</i> = 25)	(<i>n</i> = 20)
		<i>P</i> = 0.37		<i>P</i> = 0.73

The table shows the percentages of mice that exhibited normal signs. Body weight is expressed as mean \pm s.e.m.

Supplementary Table S3. General condition, neurological reflexes, and body weight of *Tsc2*^{+/−} mice.

	Male WT (n = 24)	Male <i>Tsc2</i> ^{+/−} (n = 24)	Female WT (n = 26)	Female <i>Tsc2</i> ^{+/−} (n = 25)
Fur condition (%)	100	100	100	100
Bald patches (%)	100	100	96	100
Missing whiskers (%)	100	100	100	100
Piloerection (%)	100	100	100	100
Body tone (%)	100	100	100	100
Limb tone (%)	100	100	100	100
Positional passivity (%)	100	100	100	100
Trunk curl (%)	100	100	100	100
Visual placing (%)	100	100	100	100
Eye blink (%)	100	100	100	100
Ear twitch (%)	100	100	88	96
Whisker twitch (%)	100	96	96	100
Toe pinch (%)	92	83	77	88

Righting reflex (%)	100	100	100	100
	19.9 ± 0.4	19.7 ± 0.3	17.0 ± 0.2	17.2 ± 0.2
Body weight (g)				
	(n = 27)	(n = 29)	(n = 27)	(n = 25)
		$P = 0.75$		$P = 0.57$

The table shows the percentages of mice that exhibited normal signs. Body weight is expressed as mean \pm s.e.m.

Supplementary Table S4. mRNA quantification in *Tsc2*^{+/−} brains.

Genotype	WT		<i>Tsc2</i> ^{+/−}	
	Treatment	Vehicle	Rapamycin	Vehicle
<i>Tsc1</i>	1.00 ± 0.089	1.05 ± 0.046	1.79 ± 0.13**	0.94 ± 0.032##
<i>Tsc2</i>	1.00 ± 0.065	1.05 ± 0.029	0.67 ± 0.035**	0.70 ± 0.017
<i>Gsk3b</i>	1.00 ± 0.052	1.00 ± 0.029	0.98 ± 0.011	1.01 ± 0.036
<i>Mapk1</i>	1.00 ± 0.067	1.00 ± 0.068	0.94 ± 0.029	1.03 ± 0.062
<i>Ulk1</i>	1.00 ± 0.080	1.01 ± 0.096	0.98 ± 0.033	1.08 ± 0.069
<i>Depdc6</i>	1.00 ± 0.11	1.02 ± 0.11	1.04 ± 0.067	1.11 ± 0.073

The levels were standardized to β-actin and are expressed relative to the levels of vehicle-treated WT mice (mean ± s.e.m). ** $P < 0.01$, compared with vehicle-treated WT mice (two-tailed t -test); ### $P < 0.001$, compared with vehicle-treated *Tsc2*^{+/−} mice (two-tailed t -test).

Supplementary Tables S5. Densitometric analysis of total S6K and Akt.

Genotype	WT		<i>Tsc2</i> ^{+/-}	
	Vehicle	Rapamycin	Vehicle	Rapamycin
total S6K	1.00 ± 0.082	0.938 ± 0.040	1.03 ± 0.043	0.978 ± 0.041
total Akt	1.00 ± 0.058	0.882 ± 0.033	0.934 ± 0.044	0.981 ± 0.026

The levels were standardized to β -actin and are expressed relative to the levels of vehicle-treated WT mice (mean ± s.e.m).

Supplementary Table S6. Details of primers and probes used for quantitative RT-PCR.

Gene symbol	Accession#	Primer	Primer	Universal ProbeLibrary
		(forward, 5' to 3')	(reverse, 5' to 3')	Probe#
Depdc6	NM_145470.2	cgcattggctgaaggcctagt	ttagatggtggccgttatctt	108
Eef2k	NM_007908	gctccttggatccttc	gcagcaaggatggcctc	74
Gsk3b	NM_019827.5	ttctacaggacaagcgatttaaga	cggactatgttacagtggctagc	50
Mapk1	NM_011949.3	gacagagtacgtagccacacgtt	agccccacagaccaaataatcaa	50
Tsc1	NM_022887.3	gctggaggggactgtgaggta	gcctggtatcttcatggtca	52
Tsc2	NM_011647.2	cgcagcatcagtgtatctgaa	cgtcgtaaggatgtctgt	84
Ulk1	NM_009469.3	ggatccatggtgtcaactgc	caaggcgagctgattgtacc	73